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Plasma Cholinesterase Activity in Patients with Uterine Cervical Cancer during Radiotherapy

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ABSTRACT

The objectives of this study were to investigate: 1) the activity of pseudocholinesterase (PChE) in patients with uterine cervical cancer in different stages (uterine cervical carcinoma in stages II b and III and recurrent cervical carcinoma in stages III and IV a,b) and to compare it to the enzyme activity in patients with benign tumour of the uterus, and 2) the effects of radiotherapy on enzyme activity in those patients with uterine cervical carcinoma for which the chosen treatment was radical radiotherapy. Thirty patients with uterine cervical carcinoma in stages II b and III (Group A), sixteen patients with recurrent cervical carcinoma in stages III and IV a,b (Group B) and thirty-eight patients with benign tumours of the uterus (control, Group C) were evaluated and their PChE activity was determined prior to any treatment (pre-therapy enzyme activity). All eighty-four patients were free of any liver disease. The results have shown that the patients of Group A had the pre-therapy PChE activity practically identical to those in group C, but patients of Group B had significantly lower values of PChE with respect to enzyme activities of Groups A and C ($p < 0.001$). That is to say, PChE activity was influenced by the extent to which the malignancy had spread. Radical radiotherapy (up to 8 weeks in doses higher than 50 Gy into point A; average 80 Gy) which was the chosen treatment only for patients from group A did not cause a significant inhibition of PChE activity in any patients in comparison with their control values. With regard to the role of PChE in hydrolysis of succinylcholine, our results about the influence of the malignant disease and the radiotherapy on PChE activity are clinically significant.

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Introduction

Two types of cholinesterases have been recognized in mammalian systems^{1,2}. The first is true cholinesterase (acetylcholinesterase, AChE; Enzyme Commission Number: EC 3.1.1.7), which is responsible for hydrolysis of acetylcholine at the neuromuscular junction and is found in the nervous system and in red blood cells^{1–4}.

The second is pseudocholinesterase (plasma cholinesterase, PChE, ChE, butyrylcholinesterase, BuChE; Enzyme Commission Classification Number: EC 3.1.1.8.). This is a serum esterase classified on the basis of its preference for butyrylcholine as a substrate rather than for other esters of choline^{1,2,5,6}. PChE, like albumin, is synthesised in liver cells and released into plasma immediately following its synthesis^{7,8}. The PChE concentration in plasma is therefore a reflection of the rate of its formation in hepatocytes, and any alteration of its activity can be an indication of cellular impairment^{9,10}. The true physiological function of PChE is still unknown. Consequently, it is considered to have an essential role in the transmission of slow nerve conduction, or in the regulation of plasma choline levels^{1,2} or in serum lipid and lipoprotein metabolism^{12–15}. From the pharmacological point of view, this enzyme is responsible for rapid hydrolysis and short duration of action of succinylcholine^{16,17} and the ester type of local anaesthetics¹⁸. A significant reduction in PChE activity may prolong muscle relaxation during surgery. For a practical reason it is of importance to determine whether the reduction in PChE activity is inherited (rare genetic variant of atypical or dibucaine-resistant enzyme) or acquired^{19,20}. The frequency of the acquired reduction in enzyme activity is significantly higher than in the inherited reduction and it could be a consequence of decreased enzyme syn-

thesis due to liver disease (i.e. acute hepatitis or hepatic metastasis)^{9,21,22} or malnutrition²³, carcinoma²⁴, uremia²⁵, treatment with glucocorticoids^{28,29}, ranitidine³⁰, oral contraceptives with oestrogens³¹, etc.

Generally, there are very few data about the influence of radiotherapy on enzyme activity of AChE³² or PChE. The results of some investigators show that X-ray therapy in patients with malignoma was followed or by a poor reduction of PChE activity³³, or that the influence of X-ray therapy on PChE activity is directly related to signs of disease and enzyme activity observed prior to treatment³⁴.

With regard to the clinical importance of PChE in anaesthesia, and the high incidence of uterine cervical carcinoma as a malignant disease, the aims of this study were to determine plasma cholinesterase activity in patients with uterine cervical carcinoma in stages II b and III and patients with recurrent cervical carcinoma in stages III and IV a,b and to compare their values with the enzyme activities of patients with benign tumour of the uterus. Another aim of the study was also to investigate the effect of radiotherapy on PChE activity in patients with cervical carcinoma for which this therapy was treatment of choice.

Patients and method

Total number of patients included in this study was 84 and they were divided into three groups. Forty-six patients with uterine cervical carcinoma in different stages were in group A and B and 38 patients with benign tumours of uterus were included in control, group C. No patients in Group A and C had a history of carcinoma and no patients in all three groups were treated with glucocorticoids, oral contraceptives, oestrogens, barbiturates and sympatolytic drugs for at least 1 month before the beginning of our in-

vestigation. Pregnant women were also excluded. The study started in January 1992 and was closed in April 1995. The Ethics Committee of the School of Medicine, University of Zagreb, Croatia approved the study.

Group A consisted of 30 patients with uterine cervical cancer stages II b and III (FIGO classification, 1988.) at initial diagnosis. Their performance status (ECOG) was 0–1. The treatment for all of them was the same, i.e. radical radiotherapy, and most of them received radiation treatment up to 8 weeks (average 6 weeks) in doses greater than 50 Gy (average 80 Gy) into point A.

Group B consisted of 16 women with recurrent cervical cancer in stages III and IV a,b of the disease (FIGO classification 1988.) that received prior radiotherapy (2–6 years before). Their performance status (ECOG) was 2–3. They were free of metastatic liver disease, but most of them had heavily impaired kidney function. The treatment for all patients from Group B was not the same (i.e. palliative and/or symptomatic), and for this reason, all patients from Group B were excluded from further investigation.

Control, Group C consisted of patients with benign tumour of the uterus and who were treated only surgically.

PChE monitoring

In all patients from Group A blood samples for the measurement of PChE were taken once prior to the beginning of radiotherapy (i.e. after admission to the hospital), and twice a week during radiotherapy. In all patients from Groups B and C blood samples for measuring PChE activity were taken only once after admission to the hospital.

Enzyme activities measured in all patients after admission to the hospital (i.e. before any treatment for all three Groups)

represent their pre-therapy enzyme activities.

Since the atypical or dibucaine-resistant enzyme in European population is very rare, we did not determine dibucaine phenotype in our patients.

Measurement of PChE activity

Blood was centrifuged at 1400 g for 10 min and stored at -20°C until analysed. PChE activity was determined by the method of Ellman et al. using butyrylthiocholine as substrate³⁵. Enzyme activity is expressed as μmol of substrate hydrolyzed per min per ml of plasma. Under standard conditions and using the Ellman reaction and butyrylthiocholine as substrate, the range of values of enzyme activities for normal healthy individuals is 2.5–6.7 or 2.1–5.25 $\mu\text{mol}/\text{min}/\text{ml}$ ^{36,37}.

Statistical analysis

Data are shown as mean standard deviation of the mean.

Results were analysed using unpaired and paired Student's test, Friedman analysis of variance and Wilcoxon pairs test. P value under 0.05 was taken as significant.

Results

Pre-therapy enzyme activity

The enzyme activities measured in all the patients from Groups A and B and Group C after admission to the hospital, i.e. before any treatment are presented in Table 1. The average age of each Group was as follows: Group A 51.6 \pm 12.7 years, Group B 56.8 \pm 10.3 years and Group C 45.2 \pm 10.3 years.

The mean values of PChE activity in patients from Group A and B were 4.36 \pm 1.30 and 2.46 \pm 1.24 $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{ml}^{-1}$ respectively. A significant difference in the enzyme activity existed between Group A and Group B ($p < 0.001$); i.e. the patients

TABLE 1
PChE ACTIVITY IN PATIENTS BEFORE ANY TREATMENT
(PRE-THERAPY ENZYME ACTIVITY)

Values are expressed as mean SD

Group	Number of Patients	Age (yr.)	Activity of PChE mol. min ⁻¹ .ml ⁻¹
A	30	51.6 12.7	4.36 1.30
B	16	56.8 10.3	2.46 1.24*
C (control)	38	45.2 10.3	4.46 1.19

* Indicates a statistically significant difference of the PChE value when compared with the Group A and Group C ($p < 0.001$)

TABLE 2
PChE ACTIVITY IN PATIENTS OF GROUP A DURING
RADIOTHERAPY UP TO 8 WEEKS

Values are expressed as mean SD

Week of Therapy	Number of Patients	Activity of PChE mol. min ⁻¹ . ml ⁻¹
Before treatment	30	4.36 1.30
Treatment		
1 st	30	4.31 1.26
2 nd	30	4.22 0.92
3 rd	30	4.36 0.99
4 th	30	4.44 0.99
5 th	27	4.35 0.99
6 th	24	4.90 0.99
7 th	13	4.22 0.79
8 th	5	3.92 1.30

from Group B had significantly 1.7 times lower values of PChE activity in comparison with Group A. The same statistically significant difference was noted between the mean values of PChE activity of patients from Group B and control group C ($p < 0.001$) (Table 1). But there was no significant difference in the PChE activity between Group A and the Group C.

Enzyme activity during radiotherapy

PChE activity in 30 patients from Group A with uterine cervical cancer stages II b and III during radiotherapy is shown in Table 2.

The activity of PChE did not significantly change in all 30 patients from Group A, i.e. radical radiotherapy in these patients with total radiation doses higher than 50 Gy during 4–8 weeks exerted no significant effect on PChE activity in comparison with their control values ($p > 0.05$) (Table 2).

As shown in Table 2, the average duration of radiotherapy in 80% of the patients was 6 weeks. That is the main reason why the number of patients after that time was less than 50%. So, although the number of patients in the 7th and 8th week

of radiotherapy was 13 and 5 respectively, in comparison with the number at the beginning of therapy, the drop in the activity in the 8th week was only about 10%.

Discussion

This study has shown that all patients had plasma cholinesterase activity within the normal range before the beginning of any therapy and that all patients with recurrent cancer (group B) had statistically significant lower values than Groups A and C. Our results have also shown that radiotherapy in patients from Group A did not affect the plasma cholinesterase activity.

With regard to the range of values of enzyme activity for normal healthy individuals that were mentioned earlier, it can be seen that the PChE values of patients from Group A were near the upper limit of enzyme activities for healthy persons. On the other hand, mean value of PChE activity in patients from Group B (recurrent cervical cancer in stages III and IV a, b) was on the lowest limit of normal values. We suppose that significant difference in PChE activity between the patients of Groups A and B before the any therapy was due to the poor general condition and advanced malignant disease in all patients from Group B. On the other hand, our results have shown a similar direct relationship between the PChE activity and the degree of malignant disease, as it was found by other investigators. Thus, Kaniaris. et al. determined PChE levels in 180 patients with carcinoma and in 146 normal subjects²⁴. Serum cholinesterase activity was significantly lower in patients with malignoma than in normal controls, though still within normal range. Kaniaris et al. also concluded that the degree of serum PChE activity inhibition was influenced both by the extent to which the malignancy had

spread and by the site of the primary carcinoma. Low PChE activity was also found in 118 of 2215 surgical patients³⁸ and out of 118 patients, 23 (19.5%) had malignancies (non-metastatic or metastatic). Kreuscher H. et al. compared the activity of PChE between normal patients and patients with malignant tumors or biliary obstruction and found that due to tumors or inflammation those patients had a significant decrease of enzyme activity³⁹. All these examples clearly show that the only reason for significantly lower PChE activity in our patients from Group B is the advanced malignant disease. Other factors, like the time of blood sampling, or intake of food and drink do not seem to be important.

It is mentioned that in patients with a significant reduction of PChE activity the effect of succinylcholine may be prolonged irrespective of their PChE phenotype^{26,27,31}. So, Viby-Mogensen and Hanel reported an episode of prolonged apnoea after suxamethonium in 14 patients who showed a low activity of the usual type of enzyme⁴⁰. According to their results, the low PChE activity due to an acquired deficiency, i.e. liver disease, chronic debilitating disease and carcinoma, was established in 12 out of the 14 patients. In our investigations, PChE activity in all 16 patients from Group B (recurrent cervical cancer in stages III and IV a, b) after admission to the hospital and before any therapy was also significantly lower in comparison with subjects without malignoma (Control, Group C) or with malignoma in the early stage of disease (Group A).+ Although we did not determine the phenotype of PChE, we supposed that most patients from all groups had the usual phenotype, because the frequency of atypical phenotypes (i.e. atypical homozygotes and heterozygotes) in the European population is practically very low (< 5%)⁴¹. For this reason we consider that hydrolysis of succinylcholine in

patients with uterine cervical cancer in advanced stages of the disease and with the low acquired PChE activity due to malignancy may be prolonged as well. These facts about the influence of advanced stages of the malignant disease on PChE activity are important for the anaesthesiologist in case a planned or emergency surgery in these patients is required.

Our results have shown that radiotherapy up to 8 weeks in patients from Group A did not significantly decrease the PChE activity and it was practically similar to the control value all the time. The results of mean enzyme activity measurement in 27–30 patients from Group A during the 4–6 weeks of radiotherapy show that the decrease in PChE activity was approximately 5% compared with their control value. Also, a decrease of PChE activity in 5 patients from Group A who were on radiotherapy during 8 weeks was between 0–10% (Table 2). Consequently, any relationship between the PChE activity and the dose and duration of radiotherapy does not exist. It is known that the half-life of PChE in plasma is in the 8–12 days range². In our results, values of the PChE activity during radiotherapy were practically unchanged and it is not probable that the radiotherapy in patients with early stage of disease had any effect on the enzyme synthesis in the liver or on its activity in plasma.

Our results are similar to those obtained by Guminska et al³⁴, where the activity of PChE was measured in 21 pa-

tients with Hodgkin's disease in different stages. These results have shown that the least changes in enzyme activity in the course of X-ray therapy were observed in the group of patients who had the least-advanced signs of disease. These changes in PChE activity were not significant. But the patients with the greatest progress of the disease had a decrease in cholinesterase activity observed prior to treatment and which intensified during X-ray therapy. The possible reasons are probably the poor general condition of patients and/or the radiotherapy.

In conclusion, patients with early stage of uterine cervical carcinoma have the same values of PChE activity as patients with benign tumour of the uterus, while patients with uterine cervical carcinoma in advanced stage have significantly very low values of enzyme activity in comparison with patients in the early stage of the disease, or control group. In patients with the early stage of malignant disease (uterine cervical carcinoma in stage II b and III) treatment with radiotherapy did not significantly affect the activity of PChE. Since the pseudocholinesterase is of clinical importance in hydrolysis of succinylcholine, these results also have clinical significance for the anaesthesiologist.

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AKTIVNOST KOLINESTERAZE U PLAZMI BOLESNICA TIJEKOM RADIOTERAPIJE RAKA VRATA MATERNICE

SAŽETAK

Ciljevi ovog istraživanja bili su: 1) u pacijentica s rakom vrata maternice u različitim stadijima maligne bolesti utvrditi aktivnost pseudokolinesteraze (PChE) i usporediti s aktivnosti istog enzima u pacijentica oboljelih od benignog tumora maternice; 2) u pacijentica s rakom vrata maternice u kojih radioterapija predstavlja izbor liječenja ispitati učinke radioaktivnog zračenja na aktivnost PChE. Istraživanje je provedeno na 30 pacijentica s rakom vrata maternice II b i III stadija bolesti (Grupa A), na 16 pacijentica s rekurentnom malignom bolesti III i IV a,b stadija (Grupa B) te na 38 pacijentica s benignim tumorom maternice (kontrola, grupa C). Niti u jedne pacijentice nije dokazano oštećenje funkcije jetre. U svih 84 pacijentica aktivnost PChE prvi puta izmjerena je neposredno nakon primitka na liječenje (aktivnost enzima prije početka bilo kakve terapije). Rezultati mjerenja pokazali su da je aktivnost PChE u pacijentica iz grupe A praktički identična aktivnosti enzima pacijentica iz Grupe C, dok su pa-

cijentice iz grupe B (rekurentna maligna bolest) imale značajno niže kontrolne vrijednosti enzima i u odnosu na one iz Grupe A i u odnosu na pacijentice iz skupine C ($p < 0,001$). Očito je da proširenost malignog procesa utječe na aktivnost enzima. Postupak radioterapije (duljina terapije do 8 tjedana, doze veće od 50 Gy u točku A; prosječno 80Gy) odabran je samo za pacijentice Grupe A. Rezultati mjerenja aktivnosti PChE tijekom postupka radioterapije pokazali su da niti u jedne pacijentice iz grupe A nije došlo do značajnog pada aktivnosti PChE u usporedbi s njihovim kontrolnim vrijednostima. Obzirom na ulogu koju pseudokolinesteraza ima pri razgradnji suksinilkolina, naši rezultati o utjecaju maligne bolesti i radioterapije na aktivnost enzima imaju kliničku važnost.